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## **CLAIMS**

- 1. An assay method for TSH-R auto-antibodies or TSH, which method includes the step (a), which is:
- contacting a test sample, in the presence or absence of TSH, with cells from a clone expressing human TSH-R stably transfected with a reporter construct comprising cDNA of both
  - (i) a reactant, such as an enzyme, capable of causing a measurable response when brought into contact with a corresponding substrate, such as a protein, and
  - (ii) a promoter containing cyclic AMP (cAMP) response elements (CREs),

whereby levels of the reactant vary with induced endogenous cAMP levels.

- 2. An assay method according to claim 1, wherein the promoter comprises a promoter sequence or synthetic oligonucleotide which contains the CRE consensus sequence, TGACGTCA.
- 20 3. An assay method according to claim 1 or claim 2, further including the step (b), which is:

  adding the corresponding substrate to cells thus contacted.
  - 4. An assay method according to claim 3, further including the steps:
- (c) measuring the response in the cells exposed to the substrate; and
  - (d) comparing the response from test step (c) with the response from a standard or normal sample which has undergone steps (a) to (c).

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## **AMENDED SHEET**

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- 5. An assay method according to any preceding claim, wherein the promoter is that for the glycoprotein hormone alpha subunit that contains tandem cAMP response elements.
- 5 6. An assay method according to any of claims 1 to 4, wherein the promoter comprises a construct driving the CAT enzyme
  - 7. An assay method according to any preceding claim, in which the measurable response is a colour change, fluorescence change or emission of light.
    - 8. An assay method according to claim 7, wherein the reactant is selected from chloramphenicol acetyl transferase (CAT), Firefly luciferase, Renilla luciferase, β-galactosidase, alkaline phosphatase, horseradish peroxidase and green fluorescent protein.
    - 9. An assay method according to claim 4, which comprises, in step (a), the use of a luciferase cDNA driven by a promoter containing cAMP response elements; in step (b), the use of luciferin; and, in step (c), measuring the light output from the cell lysate in the presence of luciferin.
    - 10. An assay method according to any preceding claim, wherein the reporter construct comprises α-luciferase.
  - 11. An assay method according to any preceding claim, wherein the clone for use in step (a) is obtainable by stable co-transfection of CHO cells or any eukaryotic cell line with a cDNA containing the coding region of hTSH-R in a eukaryotic expression vector and a cDNA containing the reporter construct comprising both the promoter and the reactant.

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- 12. An assay method according to any preceding claim, wherein all reagents used therein are brought together in one or more steps; and/or wherein two or more of the steps (a) to (d) are carried out substantially simultaneously.
- 13. An assay method according to any preceding claim, which is carried out by manual, partly automated or fully automated means.
- 10 14. A kit for carrying out an assay according to any preceding claim.
  - 15. A kit according to claim 14, which kit comprises:
    - (a) cells from a clone expressing human TSH-R stably transfected with a reporter construct comprising cDNA of both a reactant, such as an enzyme, capable of causing a measurable response when brought into contact with a corresponding substrate, such as a protein, and a promoter containing cyclic AMP (cAMP) response elements (CREs), whereby levels of the reactant vary with induced endogenous cAMP levels;
    - (b) a standard or normal sample for the assay;
    - (c) medium for culturing and/or reconstituting the cells; and
    - (d) instructions for carrying out the assay.
- 16. A kit according to claim 15, wherein the promoter comprises a
  promoter sequence or synthetic oligonucleotide which contains the
  CRE consensus sequence, TGACGTCA.
  - 17. A kit according to claim 15 or claim 16, further comprising:
    - (e) buffer for lysing the cells; and/or
  - (f) buffer for the reporter construct, preferably luciferase buffer;

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and/or

corresponding substrate, preferably luciferin, in buffer; (g) and optionally, a luminometer.

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- A kit according to any of claims 14 to 17, wherein the reporter 18. construct comprises the plasmid pA3luc having the glycoprotein hormone  $\alpha$  subunit promoter introduced therein.
- A kit according to any of claims 14 to 18, wherein the CRE-containing 19. sequence is sub/cloned into a commercially-available luciferase 10 reporter system, such as pGEM-luc.
  - A kit according to any of claims 14 to 18, wherein the reporter 20. construct comprises a plurality of plasmids.
  - A kit according to any of claims 14 to 18, wherein the human TSH-R is 21. sub-cloned into a eukaryotic\expression vector.
  - A kit according to claim 21, wherein said eukaryotic expression vector 22. is pSVL.
  - A kit according to claim 21, wherein the TSH-R is sub-cloned into a 23. dual vector that incorporates the antibiotic resistance gene within the same plasmid.
  - A kit according to claim 23, wherein the dual vector comprises 24. pcDNAIII.
- A kit according to any of claims 14 to 24, wherein the cells for 25. component (a) are from clone JP09 as identified herein, which have 30

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been stably transfected with, in the order of, 10<sup>5</sup> TSH-R per cell.

- 26. A kit according to claim 25, wherein said cells are co-transfected with both α-luciferase cDNA and a puromycin resistance encoding plasmid.
- 27. A kit according to any of claims 14 to 26, wherein the cells are lyophilised (freeze-dried), frozen or comprised in a gel, and provided in individual containers.
- A kit according to any of claims 14 to 26, wherein said cells are further co-transfected to provide the assay with a method of correcting for the number of cells seeded in a well during use.
  - 29. A kit according to claim 28, wherein said cells are further cotransfected using a Renilla luciferase plasmid.
  - 30. An assay method or a kit according to any preceding claim for use in association with a condition or disease selected from: autoimmune thyroid disease, non-autoimmune thyroid disease, autoimmunity of non-thyroid origin and polyendocrine disease.
  - 31. An assay method or a kit according to any preceding claim for use in screening patients selected from: pregnant women, those with euthyroid eye disease, and those receiving amiodarone and/or lithium.
  - 32. An assay method or kit according to any preceding claim for measuring TSAb or TBAb, or for measuring auto-antibodies to the TSH-R having part of its sequence modified, such as by having one or more of its amino acids replaced or otherwise modified to include tags.

ALTERIAL SHEET

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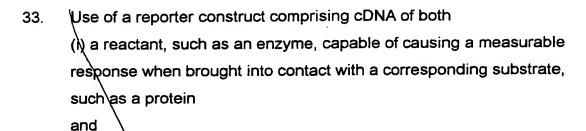
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and



(ii) a promoter containing cAMP response elements (CREs), whereby levels of the reactant vary with induced endogenous cAMP levels, which use is in an assay method or in the preparation of a kit, characterised in that said assay or kit is as defined in any preceding claim.

34. A use according to claim 33, wherein the promoter comprises a promoter sequence or synthetic oligonucleotide which contains the CRE consensus sequence, TGACGTCA.

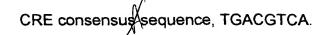
35. A use according to claim 33 or claim 34, wherein the reactant enzyme is a luciferase and/or the substrate is luciferin.

A clone expressing human TSH-R stably transfected with a reporter construct comprising cDNA of both

- (i) a reactant, such as an enzyme, capable of causing a measurable response when brought into contact with a corresponding substrate, such as a protein
- (ii) a promoter containing cAMP response elements (CREs), whereby levels of the reactant vary with induced endogenous cAMP levels.
- 37. A clone according to claim 36, wherein the promoter comprises a promoter sequence or synthetic oligonucleotide which contains the

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- 38. A clone according to claim 36 or claim 37, wherein the reactant enzyme is a luciferase and/or the substrate is luciferin.
- 39. Cells produced by a clone according to any of claims 36 to 38.

40. cDNA or mRNA expressing human TSH-R stably transfected with a reporter construct comprising cDNA of both

(i) a reactant, such as an enzyme, capable of causing a measurable response when brought into contact with a corresponding substrate, such as a protein

and

- (ii) a promoter containing cAMP response elements (CREs), whereby levels of the reactant vary with induced endogenous cAMP levels.
- 41. cDNA or mRNA according to claim 40, wherein the promoter comprises a promoter sequence or synthetic oligonucleotide which contains the CRE consensus sequence, TGACGTCA.
- 42. cDNA or mRNA according to claim 40 or claim 41, wherein the reactant enzyme is a luciferase and/or the substrate is luciferin.
- 25 43. Human TSH-R stably transfected with a reporter construct comprising cDNA of both a reactant, such as an enzyme, capable of causing a measurable response when brought into contact with a corresponding substrate, such as a protein, and a promoter containing CRE.

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